Critical switch of the metabolic fluxes by phosphofructo-2-kinase:fructose-2,6-bisphosphatase. A kinetic model

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Abstract A kinetic model for the bifunctional enzyme, phosphofructo-2-kinase:fructose-2,6-bisphosphatase, is analysed by application of the graph-theoretical method, considering comparable levels for all participants. Certain elementary reactions, distributed on the enzyme surface, are considered to be co-ordinated in a single conformational transition (a model of parallel molecular operations). The method allows us to identify in the kinetic scheme its destabilising sub-scheme as a branched cycle of elementary reactions. Under certain conditions this sub-scheme induces critical phenomena (bistability or oscillations). The computer calculations for the estimated parameter values fit well the experimental observations for this system. The model explains the periodic or bistable counterphase changes of the two opposing activities of this enzyme, observed after glucose perfusion of rat hepatic enzyme samples, and predicts drastic critical changes in kinetic behaviour induced by small external signals. The model also shows the necessity of the phosphoryl intermediate in the mechanism of the bisphosphatase for the critical kind of kinetic behaviour.

Key words: Kinetic modelling; Schematic rule; Bifunctional enzyme phosphofructo-2-kinase:fructose-2,6-bisphosphatase; Critical kind of switch; Oscillation; Parallel operation

1. Introduction

A bifunctional enzyme, phosphofructo-2-kinase:fructose-2,6-bisphosphatase (PF-2-k:Fru-2,6-P_2ase), EC 2.7.1.105: 3.1.3.46, catalyses the synthesis and degradation of Fru-2,6-P_2, the most potent activator of phosphofructo-1-kinase and inhibitor of fructose-1,6-bisphosphatase [1–4]. In this way the enzyme mediates a switching mechanism in glycolysis/gluconeogenesis opposing fluxes. The bifunctional enzyme is a homodimer, and each of its subunits contains an N-terminal kinase domain and a C-terminal bisphosphatase domain [2]. PF-2-k follows a ternary complex kinetic mechanism [2] with ATP bound first as a phosphate donor to produce Fru-2,6-P_2. The degradation of Fru-2,6-P_2 by Fru-2,6-Pase involves a ping-pong mechanism with the formation of a phosphoenzyme intermediate through phosphorylated His258 in the liver isozyme (His256 in the testis isozyme) [5]. Recent studies, using X-ray crystallography and nuclear magnetic resonance [6–8], provide further insight into the reaction mechanism. His258 is central to the catalytic mechanism of Fru-2,6-P_2ase, and the formation of a phosphoryl enzyme has been shown to be faster than the overall phosphatase activity [5].

The activities of the hepatic PF-2-k:Fru-2,6-P_2ase are reciprocally regulated by cyclic AMP-dependent protein kinase (cAMP-PK)-catalysed phosphorylation at Ser32. Phosphorylation at Ser32 inhibits the kinase and simultaneously activates the bisphosphatase [2]. Fru-2,6-P_2ase has also been shown to be inhibited by both Fru-2,6-P_2 and Fru-6-P [9]. The similar effect is induced by the inhibition of the cAMP-PK observed for the same substrates [10], so in the model we can investigate only one of these effects.

The phosphorylatory regulation by cAMP-PK is mediated through a combination of electrostatic, conformational (allosteric), and autoregulatory control mechanisms [2]. A simple kinetic model, discussed in this paper, takes into account all the enzyme properties mentioned. This model allows us to analyse the unusual kinetic behaviour of this bifunctional enzyme. The model shows its ability to switch critically (in a bistable or oscillatory manner) the metabolic fluxes in response to various external regulatory signals. The model explains the observed periodic phenomena in rat liver enzyme [11,12] and predicts some other of its unusual kinetic properties.

2. The principal kinetic scheme

The advantage of having two opposite activities on a single peptide makes it possible to co-ordinate regulation of the synthesis/degradation of Fru-2,6-P_2 by a single signal. To understand this unusual type of regulation, new kinds of kinetic models are needed other than traditional models used in enzymology.

Earlier we have discussed a principal model for this system which has predicted the critical kind of regulation with hormonal signals without external metabolic fluxes [13,14]. The predicted oscillatory behaviour of this system was then observed experimentally in rat liver enzyme [11]. In this paper a modified kinetic model, involving external fluxes and more detailed mechanisms, allows us better to understand the answer of the system to changed conditions of starvation, ischaemia, hormonal signaling, and others. Our model predicts the answer of the system with very strong changes in all ac-

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Abbreviations: PF-2-k:Fru-2,6-P_2ase, phosphofructo-2-kinase:fructose-2,6-bisphosphatase; cAMP-PK, cyclic AMP-dependent protein kinase

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tivities, which may be important for the switch of the metabolic fluxes, and explains some observed [11,12] results.

The bifunctional enzyme, E_1/E_2, catalyses the substrate cycle $S_1 \rightarrow S_2 \rightarrow S_1$. Conformational changes, which induce changes in the enzyme activities, $E_1 \rightarrow E_2 \rightarrow E_1$, have been demonstrated to correlate with phosphorylation/dephosphorylation at Ser32 [16].

We can represent the forward reaction as:

$$S_1 + ATP + E_1 \rightarrow ATP * E_1 * S_1 \rightarrow S_2 + ADP + E_1$$

Supposing ATP levels to be near saturating, we can simplify the scheme, not including ATP as a variable in the equations.

Degradation of Fru-2,6-P_2 involves a phosphoenzyme intermediate. Therefore, this reaction can be simply represented as:

$$S_2 + E_2 \rightarrow (E_2\text{-P}) + S_1;\ (E_2\text{-P}) \rightarrow E_2$$

The steady-state phosphoenzyme (E_2-P) level correlates well with the hydrolytic rate [16].

In our model we take into account the potential inhibition of cAMP-PK by both substrates, S_1 and S_2 [14]:

$$\text{cAMP-PK} + S_1 \longleftrightarrow (\text{cAMP-PK} * S_1)$$

and

$$\text{cAMP-PK} + S_2 \longleftrightarrow (\text{cAMP-PK} * S_2)$$

The principal scheme for this bifunctional enzyme, involving external fluxes to and out of Fru-6-P and regulatory enzymes PK and Ph-ase, is shown in Scheme 1.

Phosphorylation at Ser32, inducing the conformational transition $E_1 \rightarrow E_2$, and dephosphorylation at Ser32-P, inducing the reverse transition (E_2-P)$\rightarrow E_1$, are shown in Scheme 1 to be catalysed by their corresponding enzymes, PK and Ph-ase. Two different dephosphorylations, at Ser32-P and at His258-P, are shown in Scheme 1, proceeding via different pathways, (E_2-P)$\rightarrow E_1$ and (E_2-P)$\rightarrow E_2$, they can also proceed during a single conformational transition in a kinetically undivided reaction (E_2-P)$\rightarrow E_1$ (involved in the E_2 turnover, $E_2 \rightarrow (E_2\text{-P}) \rightarrow E_1 \rightarrow E_2$), which can be accelerated by the Ph-ase as well as by cAMP-PK. The advantage of the multifunctional enzyme, having multiple active groups, is the ability to correlate their activities through single conformational transitions [2]. The phosphatase, dephosphorylating Ser32-P, being unregulated by the substrates S_1 and S_2, in our model cannot be considered a separate participant in the scheme, only influencing the value of the kinetic parameter.

The detailed kinetic model for the scheme in Scheme 1 is represented by the following elementary reactions:

(a) External influx and efflux for Fru-6-P: $k \rightarrow$(Fru-6-P):$X_2 \rightarrow k_0$

(b) Forward reaction, $S_1 \rightarrow S_2$: (Fru-6-P)+(E_1):$X_1 \rightarrow k_1 \rightarrow$ (E_1):X_1+(Fru-2,6-P_2):X_4

(c) Reverse reaction, $S_2 \rightarrow S_1$: (Fru-2,6-P_2)+(E_2):$X_6 \rightarrow k_2 \rightarrow$ (Fru-6-P)+(E_2-P):X_8

The E_2 turnover can proceed via two different pathways (d,e):

(d) (E_2-P)$\rightarrow k_9 \rightarrow$($E_2$)

(e) (E_2-P)$\rightarrow k_{10} \rightarrow$($E_1$)$\rightarrow k_3 \rightarrow$($E_2$)

Pathway (e) is accelerated both by Ph-ase (k_{10}) and by PK (k_3). The process (k_{10}) involves two parallel dephosphorylations, (S32P-E_2-H258P)$\rightarrow k_{10} \rightarrow$($E_1$)+2Pi.

(f) Transition $E_1 \rightarrow E_2$ is accelerated by PK: (PK):X_5+(E_1)$\rightarrow k_3 \rightarrow$(PK)+($E_2$)

(g) PK is reversibly inhibited by both Fru-2,6-P_2 and Fru-6-P with the following parameters, respectively: k_5/k_6 and k_7/k_8. Inhibitory complexes are designated, respectively: X_3 and X_7.

Here X_1, ... X_8 denote all elementary reaction participants. Elementary reactions are enumerated by the kinetic parameters between the arrows (k_0, ... k_{10}).

The kinetic differential equations, written for this system according to the mass action law, are as follows:

$$\begin{aligned}
dx_1/dt &= k_{10}x_8 - k_3x_1x_5\\
dx_2/dt &= k - k_0x_2 - k_1x_2x_1 + k_2x_6x_4 - k_7x_2x_5 + k_8x_7\\
dx_3/dt &= k_5x_5x_4 - k_6x_3\\
dx_4/dt &= k_1x_1x_2 - k_2x_6x_4 - k_5x_4x_5 + k_6x_3\\
dx_5/dt &= -k_5x_5x_4 + k_6x_3 - k_7x_5x_2 + k_8x_7\\
dx_8/dt &= k_2x_6x_4 - k_9x_8 - k_{10}x_8\\
x_1 + x_6 + x_8 &= \text{const}\\
x_{x3} + x_5 + x_7 &= \text{const}
\end{aligned} \tag{1}$$

Two last equations represent the total levels conserved for the bifunctional enzyme, ($x_1+x_6+x_8$ = const), and for PK, ($x_5+x_3+x_7$ = const).

If the external fluxes are equal to zero (or influx is equal to efflux in the steady-state conditions), we have $k = k_0 = 0$ in Eqs. 1, and one more constraint should be added to Eqs. 1, the total substrate and product level conserved:

$$x_2 + x_3 + x_4 + x_7 = \text{const} \tag{2}$$

For simplicity we perform some calculations, supposing the elementary reaction k_{10} rapid enough. In this case X_8 is eliminated from the system with elimination of the corresponding equation for dx_8/dt from Eqs. 1, and the first equation in Eqs. 1 becomes changed to:

$$dx_1/dt = k_2x_6x_4 - k_3x_1x_5 \tag{3}$$

The conserved bifunctional enzyme level in this case becomes:

$$x_1 + x_6 = \text{const} \tag{4}$$

During the computer calculations we use the relative units for the parameters and concentrations, but we take into account the order of values, as it is known from the literature [1,2], using the Fru-6-P level one order greater than the level of the enzymes and the level of Fru-2,6-P_2.

The parameters, used in the model, are effective ones, but their values, dependent on the time scale, are chosen in realistic relations, as it is known from the literature [11,12].

The results of calculations are presented in Figs. 1–3.

Fig. 1 represents the calculated dependence of Fru-6-P and

E1 E2→(E2-P) PK Ph-ase
→Fru-6-P→Fru-2,6-P2→Fru-6-P→ E1→E2 (E2-P)→E1 (E2-P)→E2

Scheme 1. The principal kinetic scheme for PF-2-k:Fru-2,6-P_2ase.

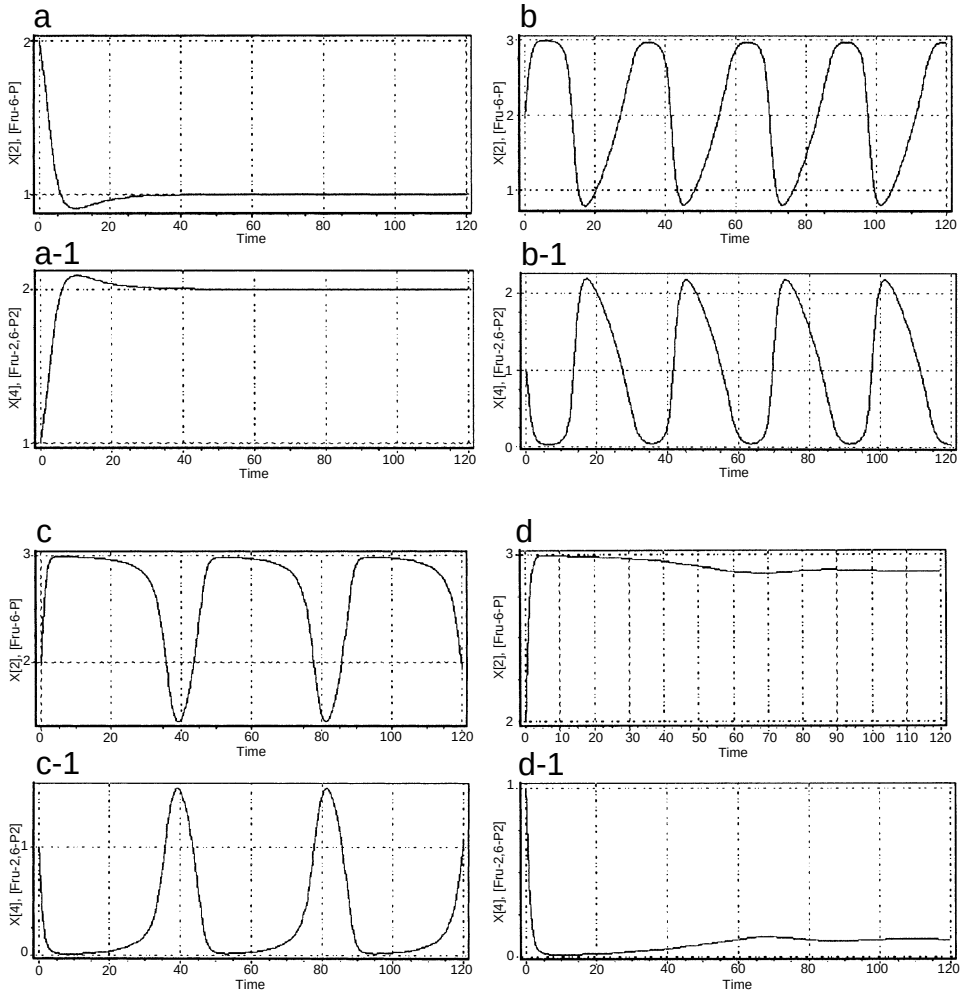

Fig. 1. a–d: Calculated dependence of Fru-6-P (X_2) level on time (relative units). Eqs. 1 are used without X_8 and without external fluxes. $x_1+x_6=0.2$, $x_2+x_4+x_3+x_7=3.3$, $x_5+x_7+x_3=0.31$. $k_1=5$, $k_2=10$, $k_5=10$, $k_6=1$, $k_7=0.5$, $k_8=0.05$. Initial values for variables: $x_1=0.1$, $x_2=2$, $x_3=0.2$, $x_4=1$. k_3 was variable: $k_3=500$ (a), $k_3=1100$ (b), $k_3=1400$ (c), $k_3=1450$ (d). a-1–d-1: Calculated dependence of Fru-2,6-P_2 level on time. Conditions as in a–d.

Fru-2,6-P_2 levels on time. These calculations were performed for the simplified Eqs. 1 without X_8, without any external fluxes, and with involving Eqs. 2–4).

The calculations show that changes in parameter k_3 strongly influence the kinetic behaviour of the system. The system undergoes two critical switch transitions and undamped oscillations, induced by the changes in parameter k_3 from $k_3=500$ to $k_3=1450$. This parameter simulates hormonal regulation of cAMP-PK, acting through the cAMP signals [1]. Other parameters are shown in the figure legends. Undamped oscillations change their frequency by changing k_3. This phenomenon can be used for coding the amplitude of the signal to the frequency in this system similarly to other systems, for those the phenomenon of such coding/decoding is well studied [17]. Moreover, this system can induce well-known oscillations in glycolysis.

Fig. 2 shows damped oscillations calculated with using the system of Eqs. 1, including X_8, but without external fluxes. For parameters and initial values see the figure legend. The simplification by elimination of X_8 (not shown in this case) does not greatly change the curve view. Therefore, this simplification is valid for other calculations presented in other

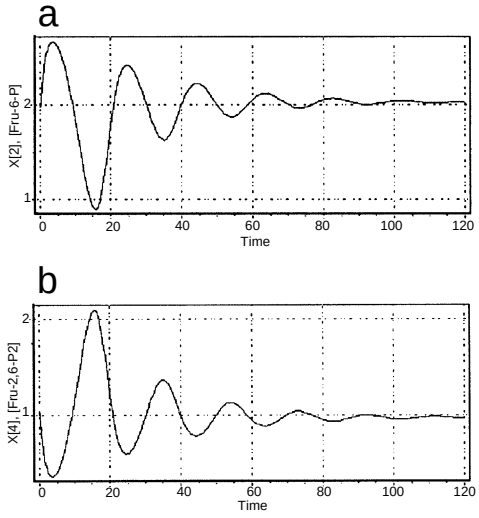

Fig. 2. Dependence of Fru-6-P (X_2) level (a) and Fru-2,6-P_2 level (b) on time (relative units), calculated according to Eqs. 1, including X_8 without external fluxes. Conditions as in Fig. 1, excluding $k_3=1100$, $k_9=0.1$, $k_{10}=10$. Initial value $x_8=0.07$, x_8 is included in the balance $x_1+x_6+x_8=0.2$.

figures. The curves in Fig. 2 describe the results, experimentally observed for the bifunctional enzyme from rat liver [11].

Fig. 3 shows the similar curves calculated for the open system (k and k_0 have non-zero values). Here X_8 is eliminated for simplicity. These curves with practically a single burst (extremal point) describe well the experimental results, published in another paper [12]. We use here the real time scale, and all the parameters can be easily adapted to the experimental results.

3. Schematic rules

We use the graph-theoretical approach [13–15], which allows us to reveal in the schematically represented system of elementary reactions its sub-system, which is sufficient to induce the critical behaviour (instability, bistability or oscillations). This approach is based on the well-known procedure of linearisation of non-linear kinetic differential equations, written according to the mass action law, such as presented in Eqs. 1, in the neighbourhood of a steady state.

The character of the solution of the non-linear kinetic equations can be estimated from the Jacobian determinant and the sub-determinants of the linearised equations. On the other hand, all the sub-determinants are induced by the schematically represented sub-systems (by the sub-schemes). Therefore, we consider sub-schemes of the kinetic scheme, involving an equal number i of different reacting substances and i different elementary reactions ($i = 1, 2, \ldots$). It is easy to show that each sub-scheme gives in the corresponding sub-determinant a term equal to its steady-state reaction rates divided by the steady-state concentrations of its substances with the sign determined by its topological structure.

For example, a sub-scheme involving three substances and three reactions gives the negative term $(v_2v_3v_5)/(x_4x_5x_6)$ in the third order sub-determinant of the linearised equations (third order Jacobian sub-determinant) for the kinetic model considered. This term should be greater in its absolute value than the positive terms, induced by other sub-schemes of the third order, to produce instability. This leads us to the simple conclusion that k_3 should be greater than k_1 for the critical phenomena to be observed. It follows from the comparison of two independent reactions, $X_1+X_2 \rightarrow 1 \rightarrow X_1+X_4$ and $X_1+X_5 \rightarrow 3 \rightarrow X_5+X_6$, involved in two different third order sub-schemes. The numbers between arrows indicate the reactions.

It is well known from linear algebra that a negative Jacobian sub-determinant can mean an unstable or oscillatory solution. Therefore, the corresponding sub-scheme, inducing the negative sub-determinant, is of interest as a potent necessary part of the critical kinetic scheme. If we find in the kinetic scheme the sub-scheme of the specific topological structure, we can say that namely this sub-system induces under certain additional conditions the critical behaviour.

In the kinetic scheme considered, where various X denote various substances, and various numbers between arrows denote various elementary reactions, the critical sub-scheme involves the typical combination of two cycles (a branched cycle), formed by the arrows: the catalytic cycle $X_5 \rightarrow 3 \rightarrow X_5$ (for the PK-catalysed reaction), and two competitive interactions $X_5 \rightarrow 5 \leftarrow X_4 \rightarrow 2 \leftarrow X_6$, also forming a cycle together with the reaction $X_5 \rightarrow 3 \rightarrow X_6$. The direction of the interactive reactions involved in the cycle is determined in our analysis as the direction of the reaction $X_5 \rightarrow 3 \rightarrow X_6$ in the same cycle, we consider only the directed branched cycles.

Competition for Fru-2,6-P_2:(X_4), interacting both with the enzyme E_2:(X_6) and with enzyme PK:(X_5), is one of the typical reasons for the critical kinetic behaviour. It has been demonstrated in our earlier work [14] that an even number of interactive reactions, $X \rightarrow * \leftarrow Y$, involved in a cycle, also involving any number of equally directed elementary reactions, $Y \rightarrow * \leftarrow Z$, is necessary to induce the critical kinetic behaviour. The branched combination of two even cycles, similar to the cycles in our model (both cycles with an even number, 0, 2, 4, ..., of interactive elementary reactions), is typical for different critical kinetic schemes. Moreover, it can be shown that a critical sub-scheme induces the critical behaviour, if no irreversible effluxes from the substances of such critical sub-scheme exist. The reversible dead-end reaction (dead-end reversible inhibition) does not eliminate critical properties in any schemes, but can stabilise the oscillations induced by the critical sub-scheme. The critical sub-scheme of the model discussed involves three substances, X_4, X_5, X_6, and three reactions, 3, 2, 5. No irreversible effluxes go out of these substances, therefore, this schematic construction can induce the critical kind of kinetic behaviour under certain values of the kinetic parameters.

For the bifunctional enzyme discussed it means that the bisphosphatase in state E_2 cannot be transformed directly to the kinase E_1. This is why the stable phosphoryl form of the bisphosphatase, E_2-H258-P, which can only be transformed to E_1 (being dephosphorylated by the specific phosphatase), is very important in the kinetic mechanism. Moreover, we suppose two different dephosphorylation processes, (E-H258-P) $\rightarrow$ E+P_i and (S32P-E) $\rightarrow$ E+P_i, can proceed in a parallel way during a single conformational transition, promoted by the specific Ph-ase.

Two dephosphorylation processes are co-ordinated in our model through a single conformational transition. Only in this case can the critical kinetic behaviour be observed.

The calculated curves describe well the effect of glucose on

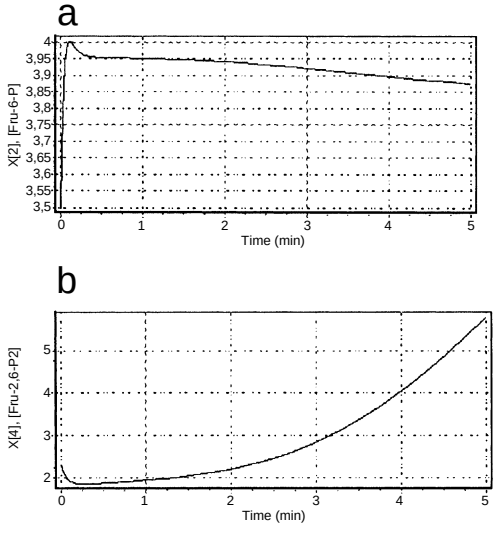

Fig. 3. Dependence of Fru-6-P (X_2) level (a) and Fru-2,6-P_2 level (b) on time (relative units), calculated according to Eqs. 1 without X_8 with external fluxes. $x_1+x_6 = 0.2$, $x_5+x_7+x_3 = 0.3$, $k_1 = 4$, $k_2 = 20$, $k_3 = 1000$, $k_5 = 10$, $k_6 = 1$, $k_7 = 0.5$, $k_8 = 20$, $k_0 = 24$, $k = 95$, $x_1 = 0.1$, $x_2 = 3.5$, $x_3 = 0.2$, $x_4 = 2.3$, $x_5 = 0.1$. Time is shown in minutes.

hepatic Fru-6-P and Fru-2,6-P_2 in starved rats [12]. Perfusion with glucose in the rat liver immediately induced hexose-P and Fru-6-P, but Fru-2,6-P_2 increased after a lag period [12], as is also obtained in Fig. 3. Under ischaemia conditions glucose perfusion was shown [11] to induce damped oscillations, similar to those shown in Fig. 2. Both substrates, X_2:(Fru-6-P) and X_4:(Fru-2,6-P_2), were observed, oscillating in counterphase [11]. Fig. 1 shows the calculated influence of the cAMP-PK level on the X_2 and X_4 time dependence. Small changes in PK level can induce strong changes in bifunctional enzyme activity.

4. Discussion

The schematic rules discussed allow us easily to analyse various kinetic models to predict their critical behaviour induced by destabilising combinations of elementary reactions. These destabilising combinations of elementary reactions are schematically represented by a branched cycle, each branch involving an even number (0, 2, 4, ...) of interactive reactions (presented each by two oppositely directed arrows in the kinetic schemes) and any number of equally directed reactions (presented each by two arrows, pointing in the same direction). An example of such schemes is considered for the bifunctional enzyme PF-2-K:Fru-2,6-P_2ase, its critical kinetic behaviour is of importance for the critical switch of glycolysis/gluconeogenesis. Oscillations of two substrates in counterphase, observed for this system [11], are simply interpreted by the model discussed. The model also explains a critical switch of metabolic fluxes by switching between two stable alternative steady states under small changes in the hormonal signal.

The schematic approach allows us also easily to estimate the parameter values needed to observe the critical phenomena.

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